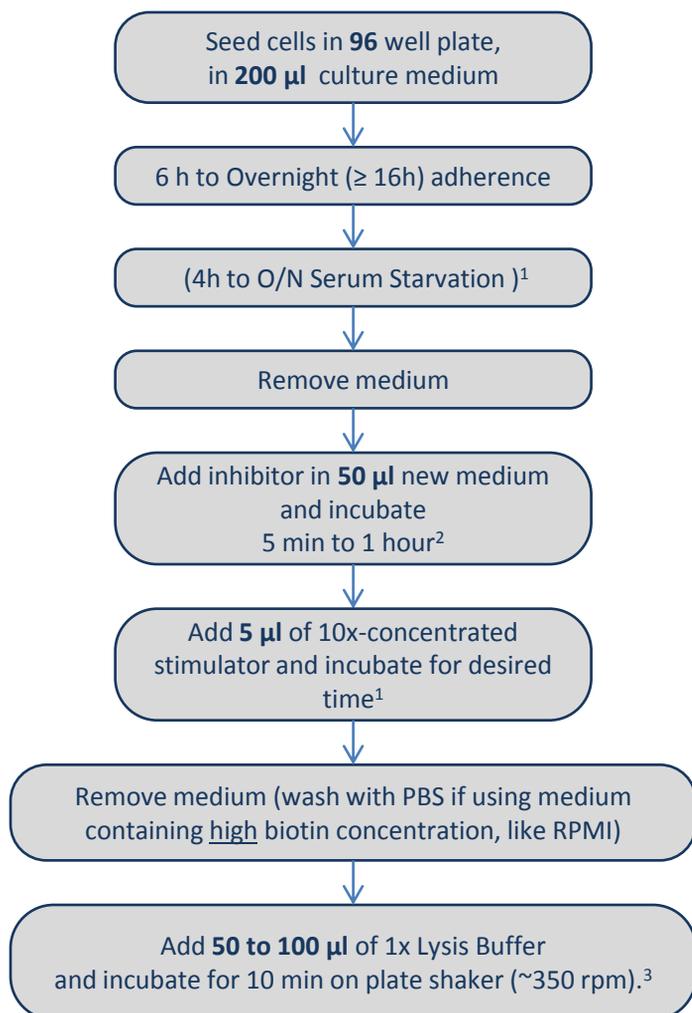
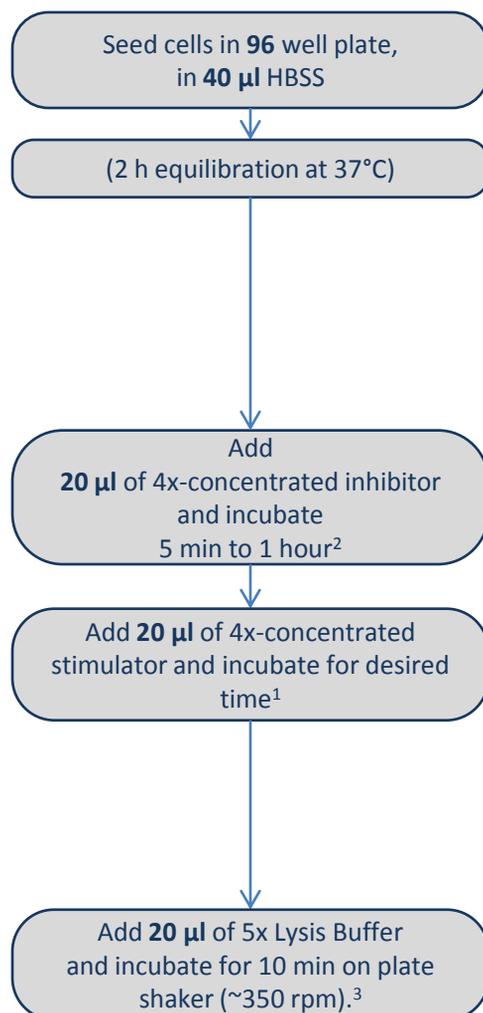


AlphaScreen® SureFire®: High Volume (HV) kits assay flowchart

Adherent Cells



Suspension Cells



Transfer **30 µl** of lysate (or control lysate) to white 96-well half-area assay plate

Add **10 µl** of **Acceptor Mix**

Seal, wrap in foil, and shake 1-2 minutes on plate shaker, Then incubate ≥ 2 h (RT° or 22°C)

Add **10 µl** of **Donor Mix**

Seal, wrap in foil, and shake 1-2 minutes on plate shaker, then incubate ≥ 2 h, up to overnight (RT° or 22°C)
Allow to equilibrate to plate reader temperature prior to reading.

Read Plate

Acceptor Mix:	Typical volume	My volumes
Reaction Buffer	160 µL	
Activation Buffer	40 µL	
Acceptor Beads	3 µL	

Donor Mix:	Typical volume	My volumes
Dilution Buffer	194 µL	
Donor Beads	6 µL	

¹ Depending on cell type and pathway analyzed.

² Depending on type of inhibitor used: 5 min is generally enough for receptor antagonists; more time is needed to block intracellular targets.

³ May stop and freeze lysates at -20°C if desired. If doing this, re-shake after thawing to ensure homogeneity of solution.